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(54) Title: METHODS FOR PROVIDING LIQUID AND SOLID COMPONENTS OF A SAMPLE FOR USE IN ASSAY METHODS

(57) Abstract

A method for providing an aliquot of a sample comprising liquid and solid components for use in an assay method comprising contacting the sample with a sampling device configured and arranged to hold an aliquot of both liquid and solid components of the sample in proportion to the liquid and solid composition of the sample, under conditions in which the sampling device holds the aliquot of the sample and providing the aliquot for use in an assay method.

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DESCRIPTION

METHODS FOR PROVIDING LIQUID AND SOLID COMPONENTS OF A SAMPLE FOR USE IN ASSAY METHODS

Field Of The Invention

The present invention relates to the field of sample collection and delivery for use in various testing or assay methods.

Background Of The Invention

The following is a discussion of the relevant art, none of which is admitted to be prior art to the appended claims.

U.S. Patent 5,709,838 describes a sampling device that will sample a material and then deliver the collected sample to a test kit. The sample device consists of a spatula and a handle. The spatula portion can be separated from the handle so that it can be left in a test container. The device is indicated to be useful for both dry particulate samples and materials dissolved in a medium capable of evaporation.

A sampling device for hemoglobin from stool is described in U.S. Patent No. 5,460,781. The device contains a fiberous bundle capable of extracting water and therefore hemoglobin from stool samples of varying consistency while not carrying any solid material to the analytical test. The device must be placed within the stool sample at multiple points to ensure adequate sample collection.

U. S. Patent No. 4,225,557 discloses a device containing a diagnostic test strip which can be contacted directly to a stool sample and then the device covered or closed to seal in the fecal sample prior to the analysis.

Another method of delivering stool specimens is described in U.S. Patent No. 4,492,124. This method comprises use of an elongated handle and a cylindrical

coring device. The coring device is pushed into the specimen to be filled with a core sample of the specimen. The coring device is then removed from the specimen and released from the handle into a suitable container for transport and /or testing. The sample is removed by pushing a sample removal device through the coring device or washing the sample from the coring device.

U.S. Patent No. 5,543,115 discloses a sample handling device comprising an elongated shaft and a grooved sample collection means. The collected sample is collected in the channels between the grooves of the device. The device is indicated to be useful for the collection of samples of any solid or semi-solid material.

U.S. Patent No. 5,066,463 discloses a fecal examination apparatus comprising a stirring rake which is used to pick up a fecal sample which is then transferred to a hollow portion of the apparatus.

WO 97/32529 describes an apparatus for removing fecal impaction from a patient comprising a shaft with flexible spines for flattening encapsulated fecal mass.

U.S. Patent Nos. 5,238,847 and 5,215,713 disclose test kits for determination of an analyte in a pasty, coatable sample in which the sample can be coated onto a sample area with a spatula.

Summary Of The Invention

The present invention concerns methods for providing an aliquot of a sample which is made up of both solid and liquid components. The claimed sampling methods improve the reproducibility and ease of delivering a difficult specimen matrix to a variety of analytical or medical testing methods. The sampling method is compatible with a wide range of sample viscosities and solid content. The sampling methods will collect both liquid and solid components of a sample and thus collect a specimen that is representative of the original sample composition. Without a representative specimen, the agent that is to be detected may appear at a

non-representative concentration and thus possibly avoid detection or be under detected. The sampling methods may be used to deliver sample to a wide variety of testing methodologies. The analytical method can be any method that identifies or quantifies one or more components of the sample. The analytical testing methods, include but are not limited to, a simple spectrophotometric analysis, a radioisotope analysis, a chemical analysis, an immunoassay, a nucleic acid analysis, and a chromatographic technique. The testing methods may examine the sample for a variety of analytes including occult blood, *Clostridium difficile* toxins, or other infectious agents if the sample is derived from a patient or may analyze the chemical composition of the sample.

The uniform, reproducible delivery of samples, regardless of specimen consistency or viscosity, to a testing method which is made possible by the current invention represents an advantage over current sampling methods. The methods of the present invention are also simple and minimize exposure to the sample materials, as the sample remains entrapped in the sampling device until delivery to a reaction or test vessel.

In a first aspect, the invention features a method for providing an aliquot of a sample comprising liquid and solid components for use in an assay method comprising contacting the sample with a sampling device configured and arranged to hold an aliquot of both liquid and solid components of the sample in proportion to the liquid and solid composition of the sample, under conditions in which the sampling device holds the aliquot of the sample, and providing the aliquot for use in an assay method.

The present method is directed to obtaining an aliquot of a sample that has liquid and solid components, as an analyte of interest may be distributed in both the liquid and solid components of the sample. Examples of samples for which the claimed method is useful for sampling include, but are not limited to, a slurry or suspension of non-biological

origin that is to be tested for the presence of one or more potential components and a specimen of biological origin such as stool, sputum, nasal washes, nasal aspirates, whole blood, pus or other exudates, or tissue homogenate. An "aliquot of a sample" is a portion of a previously collected sample, a portion of a sample that can be taken from an *in situ* location, such as a burn or wound site or nasal passages or a portion of a sample that can be taken from a manufacturing or preparation vessel. Those skilled in the art are familiar with various means for sample collection. Those of ordinary skill in the art will be able to determine the required amount of a sample which makes up an aliquot based on the analyte to be detected and the sensitivity of the assay method employed. The assay method can be any method to detect the presence and/or amount of an analyte of interest which may be contained in the aliquot of the sample. Those of ordinary skill in the art would be able to determine the appropriate assay method for a chosen analyte of interest.

The sample is contacted with the sampling device such that the sampling device is positioned on or in the sample so as to be exposed to liquid and/or solid components of the sample and such that liquid and/or solid components of the sample become affixed to the sampling device. Movement of the sampling device, laterally or rotationally relative to the sample, may be required to insure that liquid and solid sample components proportionally representative of the sample are transferred and affixed to the sampling device. The sampling device is configured and arranged to hold an aliquot of both liquid and solid components of the sample in proportion to the liquid and solid composition of the sample, i.e., the sampling device can retain both the liquid and solid components of the aliquot of the sample, if they are present, without loss of the materials until they are transferred for use in an assay method. In proportion means that the liquid and solid components of the aliquot are

representative of the relative amounts of these components in the sample.

Once the sampling device has taken up the appropriate amount of the sample, the aliquot is provided for use in the assay method, i.e., the aliquot is removed from the sampling device for use in the assay method. Removal of the aliquot can be by directly contacting the sampling device holding the aliquot of the sample with a surface which is utilized in the assay method or by immersing the sampling device in a solution which is subsequently utilized in the assay method. The solution can be contained in a reaction vessel. The solution may enhance the extraction of the analyte from the aliquot of the sample or may contain a test reagent for use in the assay method (testing solution), or may simply represent a liquid useful for removing the aliquot from the sampling device. Transfer of the aliquot from the sampling device to the solution may be facilitated by physical processes such as stirring, agitation and/or squeezing of the sampling device within a reaction vessel.

In preferred embodiments the sampling device is a brush; the brush is a brush designed to gather cells; the sample is stool; assay method is for the detection of a *Clostridium difficile* toxin; the assay method is an optical immunoassay; the sample is a slurry or suspension of non-biological origin; sample is selected from the group consisting of sputum, nasal washes, nasal aspirates, whole blood, pus, cellular exudates, and tissue homogenates; providing the aliquot comprises submerging the sampling device in a testing solution; contacting comprises pushing the sampling device into the sample, rotating the sampling device in the sample and removing the sampling device from the sample; the aliquot is delivered to a reaction vessel; the aliquot is directly delivered to a testing surface.

A brush is a preferred sampling device, although those of skill in the art will appreciate that a sample device could be any configuration of materials that can capture and hold liquid and solid components of a sample in amounts

representative of those in the sample. A brush has bristles attached to a handle. Those of ordinary skill in the art can readily determine based on the described characteristics of a brush, i.e., the shape of the brush section, the shape of the bristles within the brush, the density of the bristles within the brush, the length of the brush section, the length of the bristles within the brush, the diameter of bristles, the material composition of the bristles, the pattern of bristle distribution within the brush section and the handle design of the brush, how to select a commercially available brush or construct a brush useful for providing an aliquot of a particular sample.

In a preferred embodiment the brush is designed to gather cells. By "brush designed to gather cells" is meant a brush that is useful for obtaining cells such as from the uterine cervical canal. These brushes are referred to as cytological brushes. For example, one such brush is the CYTOBRUSH™ cytological brush (Medscand, AB, Malmo, Sweden). There are numerous designs available for a cytological brush. Some of the designs include those described in U.S. Patent No. 3,881,464, U.S. Patent No. 4,759,376, U.S. Patent No. 5,133,361, U.S. Patent No. 5,713,369 and WO 91/16855. These brushes have been designed for sampling the cervix and endocervix for cancer screening. The brushes may be shaped fairly straight and of uniform bristle length and density. They may be contoured and shaped by modifying the length and distribution of the bristles. Or the bristles may be spiraled around the shaft of the brush. The length of the brush may vary.

A preferred use of the sampling method is for reproducible delivery of stool samples especially diarrheal stool samples, as these samples contain both liquid and solid components.

Assay methods for the detection of a toxin produced by *Clostridium difficile* are known to those who practice the art and include, but are not limited to, cytotoxicity assays, immunoassays and Optical ImmunoAssay (OIA®) assays.

Optical immunoassay describes methods that are based on the interaction of light and thin films. Light that interacts with the films undergoes some type of modulation generally a phase delay but other effects are also possible. Depending on the design of the thin film device the light may under go destructive interference and generate a visual color change or the light may undergo a change in polarization that is measurable by ellipsometry, comparison ellipsometry, fixed angle ellipsometry, or other ellipsometric methods. When a visual color change is generated a simple reflectometer may replace the eye as the detector of the color change. Thin film surfaces can also be evaluated with surface profilometry, scanning tunneling microscopy, or atomic force microscopy. Attenuation of light due to interaction with thin films may result in suppression or amplification of one or more wavelength of light, a change in intensity of monochromatic light or polychromatic light, a change in polarization of light (intensity and/or wavelength or degree of polarization), or other measurable phenomena. The attenuation could also be the result of a composite of these changes in light. The thin films may be inorganic, organic, or composite organic/inorganic films. The thin films are built upon an optical support. The optical support is selected to be compatible with the detection method to be employed in the final assay format. The thin films are selected to be compatible with the optical support, the detection method, and the other components of the assay system (such as an antibody receptive layer). The selection of materials and criteria for material compatibility are addressed in U.S. Patent No. 5,550,063, U.S. Patent No. 5,482,830, U.S. Patent No.5,541,057, U.S. Patent No. 5,468,606, U.S. Patent No. 5,639, 671 and R.M. Ostroff, et al., Clin. Chem., 44, 2031-2035, 1998.

Preferably the sample is a slurry or suspension of non-biological origin. By "slurry" is meant a liquid containing fine solid components. A "suspension" is a two phase system

consisting of finely divided solid dispersed in a liquid, such as a liquid drug formulation. By "non-biological origin" is meant from any source that is not a plant or an animal. The sample can also be any material of biological origin, preferably sputum, nasal washes, nasal aspirates, whole blood, pus, cellular exudates, and tissue homogenates.

The aliquot contained in the sampling device can be transferred to a "reaction vessel" in preparation for utilization in the assay method. Reaction vessels are containers which include, but are not limited to, polypropylene tubes, and vessels made of glass, polyethylene or quartz. Once the sample is delivered to a reaction vessel, the sample must frequently be dispersed into a testing solution. This is can be accomplished by submerging the portion of the sampling device containing the aliquot of sample in a testing solution Alternatively, the aliquot of the sample can be directly transferred to a "testing surface", which is any surface on which the assay method is carried out, e.g., a membrane based assay device for immunoassay or nucleic acid analysis, a microscope slide for fluorescence assay or microscopic evaluation, or a solid agar plate for microbial analysis.

In a further preferred embodiment the method is for providing an aliquot of a stool sample for use in an optical immunoassay for detection of *Clostridium difficile* toxin comprising contacting the stool with a sampling brush configured and arranged to hold both liquid and solid components of the stool sample in proportion to the liquid and solid composition of the stool sample, under conditions in which the sampling device holds the aliquot of the sample, and providing the aliquot for use in the *Clostridium difficile* toxin optical immunoassay.

In even further preferred embodiments the aliquot is delivered to a reaction vessel prior to carrying out the optical immunoassay; the brush is a brush designed to gather cells.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

All articles, publications and patents cited in this application are hereby incorporated by reference, in their entirety.

Description Of Preferred Embodiments

The following examples are provided for further illustrating various aspects and embodiments of the present invention and are in no way intended to be limiting in scope.

Example 1: Selection and construction of a brush

A preferred sampling device is a brush, such as cytological brush (Oosina, Englewood, NJ; Hardwood Products, Co., Guilford, ME; Medical Packaging Corp., Camarillo, CA) which is used to gather cells from the cervix. However, it will be apparent to those who practice the art that a variety of types and designs of brushes are suitable for use in the claimed methods. One of ordinary skill in the art will be able to determine which of the commercially available brushes are suitable for use with a particular sample based on the liquid and solid composition of the sample and taking into consideration the discussed brush properties. Alternatively, one of ordinary skill in the art would be able to design a suitable brush based on the sample composition and the described brush properties. In the present invention, the brush is placed directly into a sample and used to withdraw a specific quantity of sample. The brush may then be inserted into a test tube or other reaction vessel and mixed with a liquid reagent. The sample may be mixed into the solution with the brush to liberate the desired analyte and create a uniform sample matrix for subsequent testing. A primary advantage of utilizing a brush for sampling specimens is the ability of the individual bristles of the brush to entrap solids, viscous

materials and liquids between the bristles. Material is safely maintained in the brush and transferred to the appropriate reaction vessel without exposure to the person performing the sampling. The brush could be sterilized, prior to use, if the aliquot of the sample is to be taken from the body, such as a wound site or nasal passages.

A diverse range of cytological brushes are commercially available. The factors to consider in selecting a commercially available design or in designing a new brush for use in obtaining an aliquot from a sample that ranges from a solid sample with low liquid content to a sample that is primarily liquid, will be reviewed. The brush selected should consider the shape of the brush section of the device, the shape of the bristles within the brush, the density of the bristles within the brush, the length of the brush section, the length of the bristles within the brush, the diameter of bristles, the material composition of the bristles, and the pattern of bristle distribution within the brush section. Also the handle design of the brush should be considered. The handle should be long enough that the user is not likely to contact the sample. But it should also be sturdy enough to allow penetration of the sample under whatever pressure is required to penetrate the sample regardless of sample consistency without breaking. The handle must also be compatible with the reaction vessel to which the sample is delivered and allow easy mixing of sample into a reaction media. A more flexible handle may be desired if the sample is being collected directly from a wound site or the nasal cavity.

The shape of the brush section of the device defines the sample volume (aliquot) transferred to a reaction vessel. The length of the brush section, the length(s) of the bristles within the brush section, the density of the bristles, and the pattern of the bristles within the brush section determine the shape of the brush. The bristles may be oriented as a spiral through the brush section. This forms a continuous distribution of bristles. The bristles

may be oriented as discrete rows. The length of the individual bristles further defines the shape of the brush section. The bristles may be of one or more length and the length may be alternated by row or by alternating bristles, etc.

The stiffness of the bristles is dependent on the composition and diameter of the material used in production of the bristle. Most bristles in commercially available brushes are nylon. The material used to manufacture the bristles may also control the hydrophobic character of the brush. The bristles must retain some level of stiffness to assist in sample penetration and collection.

The bristles may be cut during manufacturing to include a blunt end or a sharp end. A sharp end may be advantageous as it may facilitate sample penetration and retention of sample with a more solid consistency.

The bristle density must be sufficient to create collection sites for the more fluid samples. Fluid is retained between bristles by electrostatic interactions and formation of a seal to the bristles that is stronger than air/liquid interaction. More solid samples will simply adhere due to viscosity.

The proper brush design for the particular sampling application is selected based on empirical observation of the brush performance as a sample delivery device. A range of sample consistencies and composition for a given sample type should be used in the evaluation. This will ensure that the sampling device will collect sufficient volume of the sample across all possible sample consistencies. The sample volume required is primarily determined by the analytical testing method and its inherent limit of detection. A very sensitive analytical method will require less sample volume than a less sensitive method. A very sensitive analytical method is also less sensitive to the amount of liquid reagent that may be introduced into a reaction vessel to liberate the collected sample from the brush, but the volume of test reagent(s) should be optimized

to ensure complete sample recovery with minimal added reagent(s). The test reagents may include buffers, solvents, detergents, acids, bases, detector reagents, etc. Test reagent(s) may solubilize the collected sample, simply assist in suspending the collected sample, or modify sample components for subsequent detection. Reproducibility of sample recovery should be assessed across the range of sample consistencies and at the least detectable dose of the analyte to be tested by the analytical method.

The brush selected must be compatible with the sample and test reagents. This means that the brush must not release a material into the sample or test reagents that would interfere or inhibit the analytical method. The brush must not dissolve in the test reagents unless it can be demonstrated that dissolving does not affect recovery of analyte(s) for the test method. The brush must not retain the sample (aliquot) during the removal process or bind other test reagents thereby reducing the sensitivity of the method.

Based on the above described parameters, one of ordinary skill in the art would be able to select or design a brush for use in providing an aliquot from a wide variety of samples of varying consistency for use in a variety of assay methods.

The sample collection process with a brush is very simple. The handle of the brush is grasped and the brush section is pushed into the sample to cover the brush section of the sampling device. When using a long brush section a sufficient sample may be collected without complete penetration of the sample. However, leaving brush material unexposed to sample may introduce too much sampling variability. Once the brush penetrates the sample, the handle is rotated to expose all of the brush section to the sample and improve sample collection, The brush is then removed from the sample and the aliquot of sample is transferred to a reaction vessel. In some cases, such as for direct immunofluoresence methods, the sample device may

be used to smear the sample onto a testing surface such as a microscope slide. The reaction vessel may include one or more test reagents to facilitate release of the adhering sample from the bristles of the brush. The sampling device or brush may be used to mix the test reagents and the sample. Depending on the sample the brush may need to have external agitation (e.g., stirring, shaking, deforming) to express all of the aliquot of the sample into a reaction mixture. One convenient method is to place the sampling device and reaction fluid in a pliable polypropylene tube where the user can squeeze the sides of the tube and thus deform the bristles within the brush. As the brush is deformed the aliquot of the sample is released. Once a uniform mixture is obtained the sampling device may be disposed. Disposal should meet any regulatory or environmental requirements dictated by the type of sample being analyzed. The test fluid is then available for analysis by the pre-selected analytical method.

Example 2: Use of the sampling methods

A variety of sampling situations present a diversity of sample consistency to a potential sample collection device. The following examples are illustrative of the use of the claimed sampling methods, but are not intended to be limiting. The sampling device could be used to obtain an aliquot of a sample that has been previously collected or the sampling device could be used for direct sample collection from a burn or wound site or the nasal passages for subsequent culture or analysis or for collection from a manufacturing or preparation vessel. The sampling device should not deposit fibers such as from a swab into a collection site in the body.

A manufacturing process can generate a slurry where the viscosity or solid content varies. It may be necessary to determine the composition of the slurry as a function of one or more constituents within the slurry prior to the next step in the manufacturing process or prior to disposal or

processing for disposal. The sample could be a mid-synthesis slurry from a drug manufacturing process that must be tested by an analytical method such as HPLC for the ratio of active drug components.

A soil sample can be of varying consistency depending on the moisture content and contaminant within the sample area. Thus, the sample could be a contaminated soil sample from which one or more pollutants may be extracted and analyzed to monitor decontamination procedures. The tailings from a mining site could be tested for residual precious metals or toxic materials. The mining slurry could also be tested during processing for recover rates, contaminants, etc. The sampling method could be used in the collection of the yeast paste present in the fermentation process of beer and wine. The yeast paste could be tested for contamination and/or viability of the organism. Viable materials could be returned to the fermentors for use in the next fermentation process. In drug fermentation processes, the recombinant organisms producing the drug could be sampled and evaluated for contaminants and/or viability. The sampling method could also be used to sample raw processed meats for bacterial or other types of contamination. The sampling method could be used to sample other types of processed foods and beverages, in particular baby foods, prior to final packaging or by selective sampling of final packaged goods. A preferred application is the sampling and delivery of a stool sample.

As an example of sampling issues encountered with samples of varying consistency is illustrated by stool specimens. Sampling of stool specimens is important for a number of disease states. For example, occult blood provides an indication of a colon or rectal carcinoma or pre-cancerous conditions. Safe handling and efficient delivery of stool specimens to a test method is difficult. Stool samples, especially associated with diarrheal disease, can range from well formed stools to very loose, nearly liquid in consistency. The present invention provides a

single delivery system to address sampling of the variety of consistencies found in clinical samples such that an analyte of interest that may be present in liquid and/or solid components of the stool sample are delivered to an assay method.

Sputum represents another difficult material of biological origin to sample due to variation in viscosity. Testing sputum can be very advantageous for the detection of respiratory infections. Types of respiratory infections would include tuberculous, respiratory syntial virus (RSV), and influenza. The present methods are also particularly useful for sampling sputum.

Example 3: *Clostridium difficile* toxin assay

Preferred analytes are the toxins produced by *Clostridium difficile*, a known causative agent for diarrhea in certain patient populations. The detection of the toxins may be based on standard cytotoxicity assay, immunoassays, or most preferably by the method known as Optical ImmunoAssay (OIA®) assays (see U.S. Patent No. 5,486,606, U.S. Patent No. 5,541,057 and U.S. Patent No. 5,550,063).

For the production of an Optical Immunoassay for *Clostridium difficile* toxins, the following components are required. First an optical substrate must be selected. For this assay, a reflective optical substrate of known refractive index such as silicon or glass coated with amorphous silicon is selected. Because the test is designed to be a single use, qualitative assay a visual signal interpretation is preferred. To generate the visual signal, the optical substrate must be coated with an Anti-Reflective (AR) layer. The material selected for the AR layer must be related to the optical substrate by refractive index and adjusted from the theoretical thickness (determined using standard optical calculations) for the presence of the subsequent biological layers. The AR layer may be silicon nitride, silicon oxynitride, silicon mono-oxide, zirconium dioxide, titanium dioxide, and other materials are equally

well suited to the production of this layer. In this case a vapor deposited silicon nitride was used.

Once the optical device is designed, an attachment layer of a branched structured siloxane is applied to the optical surface. This attachment layer creates an environment which favors the attachment, retention, and stability of a biological capture reagent. This capture reagent or receptive materials may be selected from any biological component that is specifically reactive with the analyte of interest. In this case a polyclonal antibody to a toxin produced by *C. difficile*. This antibody layer is applied by submerging the attachment layer coated optical device in a solution containing the antibody for a pre-set amount of time. Once removed from the coating solution the optical device is rinsed in distilled water, dried, and overcoated with a protective layer for improved stability. This completed optical device may then be broken into individual test surfaces and built into a plastic assay device. One of ordinary skill in the art following the disclosure in U.S. Patent No. 5,418,136 would be able to construct other Optical Immunoassay devices that also generate a visual signal and those that generate a signal that is read by an instrument.

The *Clostridium difficile* optical immunoassay (CdTOX A OIA) is performed as follows. A sampling brush is inserted into a stool sample and rotated to fill the bristles with fecal matter. After loading the bristles of the brush, it is removed from the sample and rotated against the wall of the specimen container to remove excess material. The brush is then transferred into a flexible polypropylene reaction tube containing 300 ul of an antibody: enzyme conjugate. The brush is used to mix the specimen and the conjugate, the tube is squeezed out and then the brush is discarded. The OIA assay is performed by transferring one or two drops of the specimen/conjugate mixture to the OIA surface and incubating at room temperature for 5 minutes. If *Clostridium difficile* toxin is present in the specimen, a

complex will form between the enzyme conjugate, the toxin and the antibody bound to the OIA surface. The OIA surface is washed with a stream of buffered salt solution and blotted dry. A drop of precipitating enzyme substrate is added to the OIA surface and incubated at room temperature for 5 minutes. If the previously described complex is present, the enzyme will interact with the substrate forming a precipitating product. The presence of the precipitating product produces a visually detectable thin film on the OIA surface.

Example 4: Use of sampling brushes

A comparison was made of four commercially available brushes of different dimensions (see Table 1) for their ability to sample a suspension. The brushes utilized were Medical Packaging Corp. (MPC) (Camarillo, CA) short and standard (std) and Oosina (Englewood, N.J.) short and standard. The brushes were used to sample a suspension that is made of proteins, particles and other materials to provide the viscosity of a sample that mimics a diarrheal sample. The sample consistency is a thick paste. Ten brushes of each type were used to sample the suspension as described above in the *C. difficile* assay. The weight of the material collected was calculated and reported by subtracting the weight of the brush alone from the loaded brush (see Table 1). The %CV represents the mean divided by the standard deviation and is a measure of sampling error. As shown in Table 1, the amount of sample (aliquot) that can be captured by a brush varies with the brush design and surprisingly the method of sample collection utilizing a brush exhibits very little sample collection variation , i.e., it results in very reproducible amounts of sample collected.

Table 1

	MPC - Short	Qosina - Short	MPC - Std	Qosina - Std
Length	12.0mm	12.0mm	19.0mm	21.0mm
Diameter-Base	6.5mm	6.5mm	6.5mm	8.0mm
Diameter-Tip	5.5mm	5.0mm	5.0mm	5.0mm
Mass (g)	0.26	0.28	0.38	0.50
	0.34	0.31	0.38	0.54
	0.29	0.32	0.38	0.64
	0.31	0.32	0.39	0.57
	0.29	0.34	0.41	0.56
	0.31	0.29	0.40	0.62
	0.31	0.30	0.36	0.52
	0.29	0.25	0.38	0.60
	0.27	0.24	0.35	0.57
	0.24	0.33	0.46	0.52
Mean	0.29	0.30	0.39	0.56
Std Dev	0.029	0.033	0.030	0.046
%CV	9.91%	11.16%	7.80%	8.11%

Other embodiments are in the claims.

Claims

1. Method for providing an aliquot of a sample comprising liquid and solid components for use in an assay method comprising:

contacting said sample with a sampling device configured and arranged to hold an aliquot of both liquid and solid components of said sample in proportion to the liquid and solid composition of said sample, under conditions in which said sampling device holds said aliquot of said sample; and

providing said aliquot for use in an assay method.

2. The method of claim 1, wherein said sampling device is a brush.

3. The method of claim 2, wherein said brush is designed to gather cells.

4. The method of claim 1, wherein said sample is stool.

5. The method of claim 4, wherein said assay method is for the detection of a *Clostridium difficile* toxin.

6. The method of claim 1, wherein said assay method is an optical immunoassay.

7. The method of claim 1, wherein said sample is a slurry or suspension of non-biological origin.

8. The method of claim 1, wherein said sample is selected from the group consisting of: sputum, nasal washes, nasal aspirates, whole blood, pus, cellular exudates, and tissue homogenates.

9. The method of claim 1, wherein providing said aliquot comprises submerging said sampling device in a testing solution.

10. The method of claim 1, wherein said contacting comprises pushing said sampling device into said sample, rotating said sampling device in said sample and removing said sampling device from said sample.

11. The method of claim 1, wherein said aliquot is delivered to a reaction vessel.

12. The method of claim 1, wherein said aliquot is directly delivered to a testing surface.

13. Method for providing an aliquot of a stool sample for use in an optical immunoassay for detection of *Clostridium difficile* toxin comprising:

contacting said stool with a sampling brush configured and arranged to hold both liquid and solid components of said stool sample in proportion to the liquid and solid composition of said stool sample, under conditions in which said sampling device holds said aliquot of said sample; and

providing said aliquot for use in said *Clostridium difficile* toxin optical immunoassay.

14. The method of claim 13, wherein said aliquot is delivered to a reaction vessel prior to carrying out said optical immunoassay.

15. The method of claim 13, wherein said brush is a brush designed to gather cells.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30142

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G0IN 33/53, 33/554, 33/543, 33/544
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 7.32, 7.9, 7.92, 7.94, 7.95, 26, 973; 436/518, 528, 531, 532, 808, 811

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, STN, MEDLINE, HCAPLUS, BIOSIS, EMBASE, WPIDS, JAPIO.
ALIQUOT, LIQUID OR SOLID, BRUSH, STOOL OR SPUTUM, CLOSTRIDIUM DIFFICILE, IMMUNOASSAY, DEVICE, METHOD, OPTICALMEASUREMENTS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,963,325 A (LENNON et al.) 16 October 1990, Abstact, Figure 1 and 2, Col. 7, lines 17-20, Col. 3, lines 32-37, page 4, line 29 to page 5, line 8.	1-3, 6-12
Y,P	US 5,965,375 A (VALKIRS) 12 October 1999, Abstract, Col. 7-9.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 FEBRUARY 2000	Date of mailing of the international search report 06 MAR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BASKAR PADMA Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.2, 7.32, 7.9, 7.92, 7.94, 7.95, 26, 973; 436/518, 528, 531, 532, 808, 811